UNIVERSIDADE FEDERAL DA BAHIA
FACULDADE DE FARMÁCIA
PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS

MARIANE DOS SANTOS GONÇALVES

EFEITO TERAPÊUTICO DO CHÁ VERDE SOBRE AS MODIFICAÇÕES HEPÁTICAS DECORRENTES DO CONSUMO DE DIETA HIPERLIPÍDICA

Salvador
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Orientador: Dr. Ricardo David Couto
Coorientadora: Drª Jairza Maria Barreto Medeiros

Dissertação apresentada ao Programa de Pós-graduação em Ciência de Alimentos da Faculdade de Farmácia – Universidade Federal da Bahia, como requisito para obtenção do grau de Mestre em Ciência de Alimentos.

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Aprovada em 24 de agosto de 2020.

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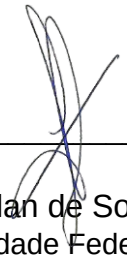

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RESUMO

O objetivo desse estudo foi avaliar o efeito da infusão de chá verde nas modificações metabólicas e hepáticas de camundongos alimentados com uma dieta rica em gordura. Camundongos machos C57BL/ 6, com idades entre quarenta e sessenta dias, foram divididos aleatoriamente em quatro grupos: controle (C, n=8), controle + chá verde (CGT, n=9), dieta rica em gordura (HFD, n=9) e dieta rica em gordura + chá verde (HFDGT, n=9). A dieta e a água (ou chá verde) foram oferecidos *ad libitum* aos animais por 16 semanas. Os seguintes parâmetros foram avaliados em todos os grupos experimentais: IMC (Índice de Massa Corporal) (a cada duas semanas); glicemia de jejum (no início e no experimento final); resposta ao TTGO – Teste de Tolerância à Glicose Oral (com 15 semanas); TTI – Teste de Tolerância à Insulina (com 16 semanas); e determinação do perfil lipídico e transaminases (com 16 semanas). Os animais do grupo HFD, quando comparados aos animais do grupo C, apresentaram maior peso corporal final (36,00 ± 1,25 g *vs* 29,87 ±1,08 g, $p \leq 0,001$), maior peso relativo do fígado (5,48 ± 0,40 g *vs* 3,71 ± 0,25 g, $p \leq 0,001$), glicemia de jejum aumentada (202,00 ± 9,78 mg/dl *vs* 142,83 ± 4,21 mg/dl, $p \leq 0,001$). Além disso, o grupo HFD apresentou outros distúrbios como intolerância à glicose ($p \leq 0,05$) e resistência periférica à insulina ($p \leq 0,001$) evidenciada pelos valores de Área Sob a Curva (ASC) de TTGO e TTI. O grupo HFDGT apresentou menor peso corporal final (32,55 ± 1,03 g *vs* 36,00 ± 1,25 g, $p \leq 0,05$), glicemia de jejum diminuída (162, 33 ±8,97 mg/dl *vs* 202,00 ± 9,78 mg/dl $p \leq 0,01$), menores níveis séricos de colesterol HDL ($p \leq 0,001$) e colesterol total ($p \leq 0,001$) quando comparado ao o grupo HFD. No grupo HFDGT, o chá verde foi eficaz na restauração da intolerância à glicose ($p \leq 0,05$), mas não teve um impacto significativo nos valores de TTI. Não houve alterações significativas nos níveis séricos de AST e ALT entre os grupos de interesse. O chá verde contribuiu para a melhora dos parâmetros antropométricos e do metabolismo da glicose, mas não houve alterações significativas nas transaminases.

Palavras-Chave: *Camellia sinensis*, catequina, DHGNA, função hepática, esteatose hepática, transaminases.

ABSTRACT

The aim of this study was to evaluate the effect of green tea infusion on metabolic and hepatic changes in mice fed a high-fat diet. Male C57BL / 6 mice, aged between forty and sixty days, were randomly divided into four groups: control (C, n = 8), control + green tea (CGT, n = 9), high-fat diet (HFD, n = 9) and high fat diet + green tea (HFDGT, n = 9). The diet and water (or green tea) were offered ad libitum to the animals for 16 weeks. The following parameters were evaluated in all experimental groups: BMI (Body Mass Index) (every two weeks); fasting blood glucose (at the beginning and in the final experiment); response to TTGO - Oral Glucose Tolerance Test (15 weeks old); TTI - Insulin Tolerance Test (16 weeks old); and determination of the lipid profile and transaminases (at 16 weeks). The animals in the HFD group, when compared to the animals in group C, had higher final body weight (36.00 ± 1.25 g vs 29.87 ± 1.08 g, $p \leq 0.001$), greater relative liver weight (5.48 ± 0.40 g vs 3.71 ± 0.25 g, $p \leq 0.001$), increased fasting blood glucose (202.00 ± 9.78 mg/dL vs 142.83 ± 4.21 mg/dL, $p \leq 0.001$). In addition, the HFD group presented other disorders such as glucose intolerance ($p \leq 0.05$) and peripheral insulin resistance ($p \leq 0.001$) evidenced by the TTGO and TTI Area Under Curve (AUC) values. The HFDGT group had lower final body weight (32.55 ± 1.03 g vs 36.00 ± 1.25 g, $p \leq 0.05$), decreased fasting glucose (162.33 ± 8.97 mg/dL vs 202.00 ± 9.78 mg/dL $p \leq 0.01$), lower serum levels of HDL cholesterol ($p \leq 0.001$) and total cholesterol ($p \leq 0.001$) when compared to the HFD group. In the HFDGT group, green tea was effective in restoring glucose intolerance ($p \leq 0.05$), but did not have a significant impact on TTI values. There were no significant changes in serum levels of AST and ALT among the interest groups. Green tea contributed to the improvement of anthropometric parameters and glucose metabolism, but there were no significant changes in transaminases.

Keywords: *Camellia sinensis*, catechin, NAFLD, liver function, hepatic steatosis, transaminases

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LISTA DE ABREVIATURAS E SIGLAS

AGL	Ácidos Graxos Livres
ALT	Alanina Aminotransferase
AST	Aspartato Aminotransferase
C	Controle
CGT	Controle + Chá Verde
DHGNA	Doença Hepática Gordurosa Não Alcoolica
EGCG	Epigalocatequina-3-galat
EHNA	Esteatoepatite não alcóolica
GT	Chá Verde
GTE	Extrato de Chá Verde
GTP	Polifenóis de Chá Verde
HDL-c	Colesterol ligado à Lipoproteínas de alta densidade
HFD	Dieta Hiperlipídica
HFDGT	Dieta Hiperlipídica + Chá Verde
LDL-c	Colesterol ligado à Lipoproteína de baixa densidade
SREBP	Proteínas Reguladoras do Elemento Esterol
TG	Triglicerídeos
TTOG	Teste de Tolerância Oral à Glicose
TTI	Teste de Tolerância à Insulina

SUMÁRIO

1. INTRODUÇÃO

As escolhas alimentares estão diretamente relacionadas à saúde da população. O aumento do consumo de calorias e gorduras saturadas, juntamente com o baixo consumo de frutas e vegetais, favorece o surgimento da obesidade e inúmeras doenças a ela relacionadas, como por exemplo, a Doença Hepática Gordurosa não Alcoólica (DHGNA) (APOVIAN, 2016; LEE *et al.*, 2020)

A DHGNA é considerada uma das consequências da obesidade e considera-se como a doença hepática mais comum nos países industrializados. Esta doença é um estado clínico caracterizada pelo depósito anormal de lipídios, principalmente os triacilglicerídeos (triglicerídeos), nos hepatócitos (POLYZOS; KOUNTOURAS; MANTZOROS, 2019). A DHGNA inclui desde a esteatose simples (compreendida como um acúmulo de gordura no fígado), até esteato-hepatite com fatores inflamatórios (EHNA), fibrose e cirrose (POLYZOS; KOUNTOURAS; MANTZOROS, 2019; ROEB, 2014).

A esteatose hepática simples pode progredir para a EHNA, com inflamação e dano celular hepático. Esta é considerada a forma de maior importância clínica, e com grande potencial de evolução da DHGNA. A EHNA pode evoluir para cirrose e trazer complicações como doença hepática descompensada e carcinoma hepatocelular (AZZAM; MALNICK, 2015; POLYZOS; KOUNTOURAS; MANTZOROS, 2019).

A EHNA está relacionada à obesidade, à dislipidemia e à resistência à insulina (CRUZ *et al.*, 2016). Na resistência à insulina ocorre o bloqueio na fosforilação do receptor, isso impede a entrada de glicose para o interior da célula. Diante desse quadro de dificuldade de utilização da glicose, cria-se um estado de hiperinsulinemia compensatória e aumento da atividade lipolítica com o intuito de utilizar a gordura como fonte alternativa de energia. Como consequência da elevação da atividade lipolítica, ocorre aumento do fluxo de ácidos graxos livres (AGL) para o fígado (LV; PATEL; ZHANG, 2019; POLYZOS; KOUNTOURAS; MANTZOROS, 2019).

No fígado os AGL podem seguir dois processos: a oxidação para fornecimento de energia alternativa (que ocorre nas mitocôndrias) ou a esterificação (com formação de triglicerídeos). Os AGL aumentam o estresse oxidativo e a desregulação da produção de adipocinas, como a IL-6, o TNFα, a leptina e a adiponectina (ROEB, 2014). Além disso, a hiperinsulinemia e a inibição da lípase lipoprotéica, resultantes da resistência à insulina, atuam de forma conjunta, ocasionando o acúmulo de triglicerídeos no citoplasma do hepatócito,

culminando com o posterior desenvolvimento da esteatose hepática (LV; PATEL; ZHANG, 2019).

É importante ressaltar que o estresse oxidativo também contribui para o desenvolvimento da EHNA. O mecanismo envolvido é bem eluciadado na literatura. As espécies reativas de oxigênio (EROs) produzidas de forma excessiva acarretam no enrolamento incorreto das proteínas no retículo endoplasmático (RE). Isso gera estresse ao RE e as e proteínas reguladoras do elemento esterol (SREBPs) que estão no RE são transferidas para o complexo de Golgi por meio de mecanismo independente. Consequentemente, as SREBPs ativa genes que estão relacionados com a esteroidogênese e a lipogênese, acarretando no desenvolvimento de doença hepática gordurosa não alcoólica (FUJII *et al.*, 2018).

Em relação aos aspectos histomorfológicos da EHNA, estes são muito similares com os encontrados na hepatite alcoólica. A lesão hepatocelular é caracterizada por hepatócitos com inflamação e fibrose. As principais alterações histológicas encontradas no parênquima hepático são: inflamação lobular, balonização dos hepatócitos e esteatose macrovesicular (LV; PATEL; ZHANG, 2019; POLYZOS; KOUNTOURAS; MANTZOROS, 2019; ROEB, 2014).

Segundo a diretriz da ESPEN 2019 (PLAUTH *et al.*, 2019), o tratamento de primeira linha para EHNA é a mudança no estilo de vida, associando dieta hipocalórica e atividade física. Uma perda de peso de 7 a 10% é recomendada para melhorar a esteatose e a bioquímica do fígado, e a redução de peso acima de 10% deve ser recomendada para melhorar a fibrose(PLAUTH *et al.*, 2019). No entanto, sabe-se que a adesão a novos hábitos alimentares é uma dificuldade relatada pelos pacientes, e novas estratégias de tratamento adjuvantes devem ser investigadas (APOVIAN, 2016).

O chá verde é uma das bebidas mais populares do mundo. É derivada da planta *Camellia simensis*, e seus principais constituintes são cafeína e catequinas (epicatequina 3-galato, epigalocatequina, epicatequina e epigalocatequina-3-galato) (CHU *et al.*, 2017; OHISHI *et al.*, 2016). Por ser rico em polifenois, pesquisadores têm investigado suas propriedades antioxidantes (BASU *et al.*, 2013), anti-inflamatórias (CAVET *et al.*, 2011; LIU; PERKINS; HENNIG, 2016), anticancerígenas (ROGOVSKII *et al.*, 2019; YANG; WANG, 2016), anti-hiperglicêmicas (LOMBO *et al.*, 2016; OTHMAN *et al.*, 2017) e antiobesidade (FRIEDRICH *et al.*, 2012; SUZUKI *et al.*, 2016). No entanto, estudos sugerem que o consumo regular do chá verde podem causar hepatoxicidade (CHAN *et al.*, 2010; EL-BAKRY; EL-SHERIF; ROSTOM, 2017; FRANK *et al.*, 2009), e essas reações hepáticas ao chá verde provavelmente podem ser atribuídas as catequinas, particularmente a epigalocatequina-3-galato (MAZZANTI; DI SOTTO; VITALONE, 2015).

Portanto, é necessária uma investigação mais aprofundada sobre os reais efeitos do chá verde sobre a função hepática, bem como avaliar se a infusão do chá verde é capaz de melhorar o perfil metabólico e hepático quando há consumo de dieta hiperlipídica.

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2. OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o efeito terapêutico da infusão do chá verde sobre as modificações metabólicas e hepáticas decorrentes do consumo de dieta hiperlipídica, bem como analisar se esses efeitos se reproduzem quando não há exposição à uma dieta com alto teor de gordura.

2.2 OBJETIVOS ESPECÍFICOS

- Investigar por meio de busca sistemática em base de dados o efeito do chá verde e suas catequinas sobre a função hepática.

- Analisar os efeitos do consumo regular de infusão de chá verde sobre a evolução do índice de massa corporal e peso final do fígado em camundongos adultos alimentados com dieta hiperlipídica.

- Avaliar em modelo animal os efeitos do consumo regular de infusão de chá verde associado a dieta hiperlipídica sobre biomarcadores séricos metabólicos (perfil lipídico e glicemia), e de lesão hepática (AST e ALT).

3. CAPÍTULO I – EFFECT OF GREEN TEA OR GREEN TEA CATECHIN CONSUMPTION ON HEPATIC FUNCTION: A SYSTEMATIC REVIEW

ABSTRACT

Objective: the purpose of this review was to assess the effect of green tea (GT) on liver function in the presence (and absence) of Non-alcoholic fatty liver disease (NAFLD). Methods: PubMed, Scopus, Science Direct, Cochrane Libary, and BVS databases were searched for articles published from January 2015 to June 2020. The research included studies performed on humans and animal model trials experimental. Results: Twenty-six articles were eligible to be included in the systematic review. Twenty articles were animal model studies, and six articles were performed on humans. The main outcomes associated with the consumption of green tea in the presence of NAFLD were: reduction in ALT and AST levels; decrease in liver lipids (TG and cholesterol); decreased macrovesicular steatosis; increased hepatic glycogen; decreased levels of inflammation markers; decreased production of hepatocins (LECT2 and SeP); increased expression of genes related to catabolism, lipolysis and β oxidation; decreased expression of genes involved in the accumulation of hepatic TG. The main outcomes associated with the consumption of green tea in the ausence of NAFLD were: hepatic inflammation, congestion of the central veins and hepatic sinusoids, hypertrophy of the hepatic arteries, and cellular infiltration (when administered a dose of 500-1000 mg). Conclusions: The use of green tea proved to be efficient as a treatment for NAFLD, with improved liver function on humans and animal model studies, but it is necessary to evaluate the dose and time of green tea administration to avoid liver damage.

Keywords: *Camellia sinensis*, NAFLD, aminotransferases, liver function, polyphenols, catechin

3.1 INTRODUCTION

Green tea is a Chinese drink derived from the *Camellia simensis* plant. It is considered one of the most popular drinks in the world. The main components present in green tea are caffeine and catechins (epicatechin 3-gallate, epigallocatechin, epicatechin, and epigallocatechin-3-gallate)[1]. Several health benefits are attributed to the catechins of green tea, as an anticarcinogenic, antifungal, anti-infective, anti-inflammatory effects[2–5]. Studies show the beneficial effects of green tea catechins on human health, and many of them are associated with improved liver function[1,2,4].

In experimental studies carried out in rodents [6-8], regardless the dose of green tea extract (GTE) used or the duration of treatment, there was a decrease in serum levels of liver enzymes (i.e.

AST, ALT), liver lipids (i.e. cholesterol, triglycerides, and fatty acids) and body mass of animals with Non-alcoholic fatty liver disease (NAFLD) [6-8].

NAFLD is characterized by the accumulation of lipids in the liver, mainly triglycerides, in amounts greater than 5% in hepatocytes. The main factors that contribute to the onset of this disease are metabolic changes, dyslipidemia, glucose intolerance, and central obesity [9-10].

However, the literature is controversial when it comes to liver disorders resulting from the consumption of green tea. In the study by El-Barky et al.[11], GTE (8.5mg/kg, orally for one month) in the mice's diet was able to induce hepatoxicity, causing liver enzymes (ALT and AST) elevation. The changes consisted of moderate centrilobular necrosis, necrotic liver cells, interstitial hemorrhage, and inflammatory cell infiltrations[11]. Therefore, further investigation into the real effect of green tea on liver function is needed.

Recently, systematic reviews have been carried out to assess the effect of green tea on liver function[12,13]. However, these reviews only evaluated the serum levels of transaminases and were limited to studies in humans. Therefore, the purpose of this review was to evaluate the effect of green tea on liver function in individuals with NAFLD and individuals without NAFLD. Besides, animal model studies were selected to understand the main metabolic pathways involved in this process

3.2 METHODS

The systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines[14]. The PROSPERO registration number is: CRD42020198281

3.2.1 SEARCH STRATEGY

A comprehensive literature search was carried out to identify relevant articles to compose this review. Electronic databases were scanned from January 2015 to June 2020 and included the following: PubMed, Scopus, Science Direct, Cochrane Library, and BVS (Biblioteca Virtual de Saúde). The following MESH terms were used: “green tea,” “catechins,” and “*Camellia sinensis*” together with “non-alcoholic fatty liver disease,” “NAFLD,” “non-alcoholic steatohepatitis,” “NASH”, "liver", "hepatic" or "liver function" and the combined phrases. It used the boolean operators AND and OR for crossing the terms. No language restrictions were imposed. Moreover, to find additional relevant articles, reference lists of related studies were also checked.

3.2.2 STUDY SELECTION CRITERIA

Potential titles and abstracts were selected by a reviewer (M.S.G.); and a second reviewer (A.P.A.M.) also independently assessed each potential article for inclusion, to determine whether it could be excluded based on the inclusion / exclusion criteria. Finally, disagreements were discussed and resolved by consensus.

Studies in humans and animals were selected according to the PICOS strategy: Participants (adult individuals with - or without - NAFLD and animal model studies with the same characteristics); Interventions (green tea treatment); Comparators (control group); Outcome (main findings related to liver function); Studie design (studies in humans and animal model). The included studies met the following criteria: 1) original article, 2) experimental studies (mice or rats) or clinical trial in adult subjects, 3) use of GT (green tea), GTE (green tea extract), or catechins/polyphenols isolated as an intervention. Articles with at least one of the following characteristics were excluded: 1) unclear data or where important information was lacking, 2) use of other food or food supplements with GT or GTE.

3.2.3 DATA EXTRACTION AND QUALITY ASSESSMENT

From the human articles, we extract the following information: first author's name, publication year, sample size, study design, country, participants' sex, age, study duration, form and administered dose of green tea, and the main outcomes related to liver function.

We extracted from the experimental studies: presence/absence of steatosis, exposure time of green tea, and green tea forms of administration, liver mass, hepatic lipids, histological analysis, and hepatic enzymes, and other information about the main outcomes related to liver function.

The quality of the experimental articles was carried out according to the guidelines ARRIVE - Animals in Research: Reporting In Vivo Experiments[15]. To evaluate the adequacy of the articles according to the guidelines, a scoring system (0 - no; 1 - yes) was used for the 20 items not listed. Articles with scores >10 were defined as high quality, and those with scores 10 were defined as low quality.

3.3 RESULTS

This search retrieved 2238 articles. After screening titles and abstracts 55 articles were selected to read the full article. Finally, 26 studies were included in the systematic review. For the assessment of liver function in the presence of NAFLD, three human studies [16–18] and 16 experimental studies were selected[5–7,19–31]. For the assessment of liver function without

NAFLD, three studies in humans[32–34] and four experimental studies[35–38] were selected (Figure 1).

3.3.1 THERAPEUTIC EFFECT OF GREEN TEA ON THE HEPATIC FUNCTION OF INDIVIDUALS (AND ANIMALS) WITH NAFLD

All studies that evaluated the effect of green tea on NAFLD patients were carried out in an Asian population (Iran[17,18] and Pakistan[16]), in both sexes, lasting twelve weeks. The

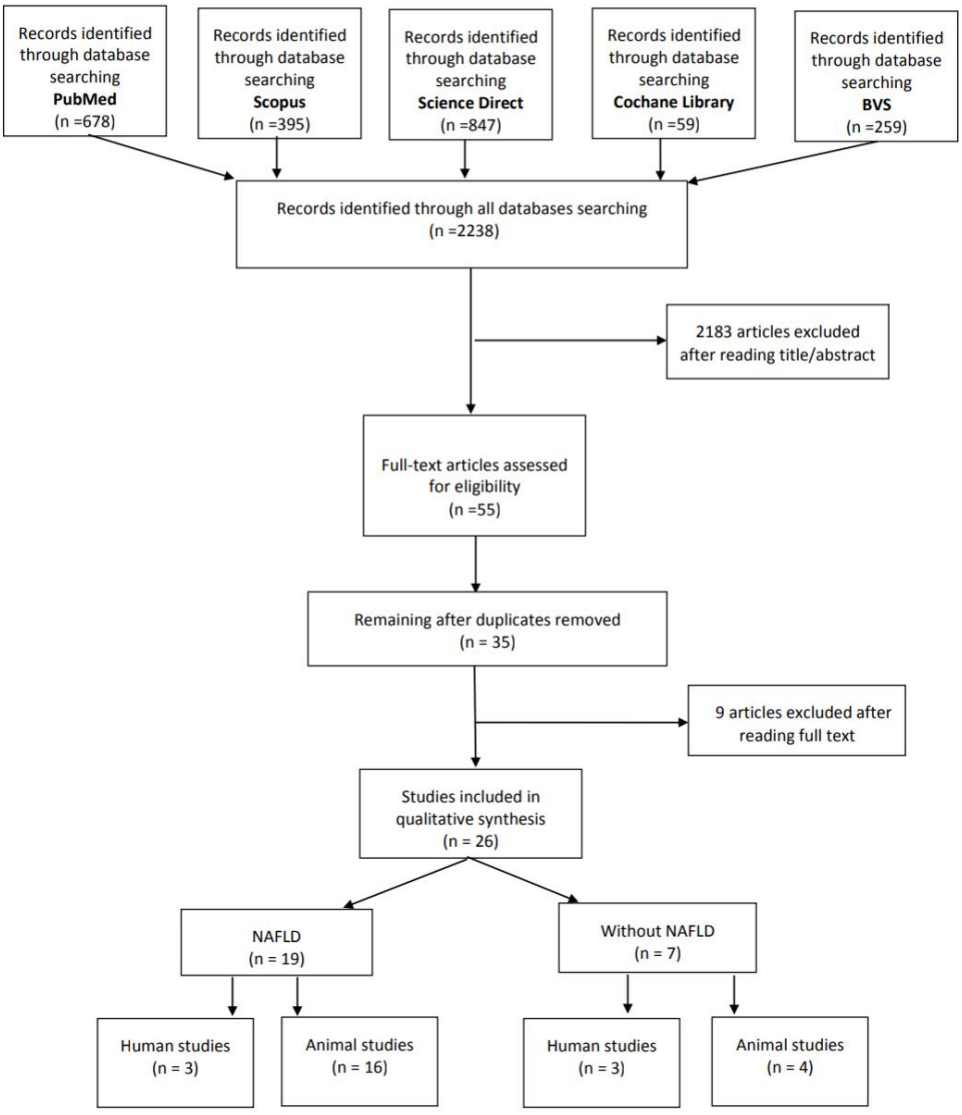

Figure 1 - Flowchart summarizing the article selection process

daily dose of GTE used ranged from 384 mg/day to 1000 mg/day. In all studies, there were a significant decrease in Body Mass Index and transaminases (ALT and AST), except for a Tabatabaee et al.[17] which the ALT decrease was not significant (Table 1).

In animal model studies, six of them used the experimental model of induction followed by intervention[7,25–29] (the mice were fed a high-fat diet for a period and then underwent GT

administration for a few more weeks), and ten studies were given green tea at the same time as the high-fat diet[5,6,19–24,30,31] (Table 2).

In the studies of the first part (Induce → Intervention), the period in which the mice received an only high-fat diet (HFD) lasted from 2 to 16 weeks, with studies with eight weeks of induction predominating[7,27,28]. The HFD used for induction contained 34 to 60% fat as an energy source, and most studies used a 60% fat diet [7,25,27](Table 2).

In the experimental model in which the animals consumed HFD in the same period in which green tea was administered (simultaneous HFD + Green Tea), we observed similar methodological characteristics[5,6,19–24,30,31]. Regarding the HFD used, studies used a diet in which the percentage of fat varied from 50 to 61%, with diets that provided 60% fat as an energy source being more prevalent[5,6,20,22,24] (Table 2).

As for the characteristics of green tea administration, 12 studies used GTE[5–7,19,20,23,24,26,27,29–31], three studies used EGCG[21,22,25], and one study used (green tea polyphenols)GTP [28]. Ten studies added green tea to the diet[5–7,19,22,24,25,27,30,31], five studies administered by gavage[20,23,26,28,29] and one study by drinking water[21]. The duration of the experiment ranged from four to 35 weeks, with more prevalent studies lasting more than eleven weeks[5,6,20–23,25,26,29,30] (Table 2).

The main outcomes associated with the consumption of green tea in animal model studies were: reduction in ALT and AST levels[5,7,24,28,30]; decrease in liver lipids (TG and cholesterol)[6,7,20,22,23,25,26,28–31]; decreased macrovesicular steatosis[5,7,26,31]; increased hepatic glycogen[26]; decreased levels of inflammation markers[24,26]; decreased production of hepatocins (LECT2 and SeP)[19]; increased expression of genes related to catabolism, lipolysis and β oxidation[20,23–28]; decreased expression of genes involved in the accumulation of hepatic TG [20,26](Table 2).

3.3.2 EFFECT OF GREEN TEA ON THE HEPATIC FUNCTION OF INDIVIDUALS (AND ANIMALS) WITHOUT NAFLD

Of the studies carried out in humans, patients without NAFLD, two were carried out in the USA[33,34], and one in the Republic of China[32]. Two studies used 2000 mg/day of GTE for 12 months[33,34]. One study used a GT drink for 12 weeks[32]. In two studies, patients had a significant increase in ALT[33,34]. In the study proposed by Yu et al.[33] patients presented a decrease in AST followed by an increase in ALT. Besides, about 5.1% of women developed moderate and severe liver function abnormalities[33] (Table 1).

Table 1 - Characteristics, methodological aspects and main results of the human studies included in systematic review

Study characteristics	Population	Characteristics of green tea intake	Comparison group (placebo)	Duration (weeks)	Main outcome	Reference/ year/ country
NAFLD Double-blind, placebo-controlled, randomized	80 patients of NAFLD; 28 years old; both sex	GTE (31.4% EGCG), capsules (500mg GTE), 2 capsule/day	Microcrystalline cellulose	12 weeks	↓BMI, ↓ALT, ↓AST GTE caused a 67.5% regression of fatty liver changes on ultrasound as compared to placebo which is 25%only	HUSSAIN et al., 2017 [19] (Pakistan)
	67 patients of NAFLD; 39.5 years old; both sex	GTE, capsules (384mg GTE), 1 capsule/day	Starch	12 weeks	↓BMI, ↓*ALT, ↓AST	TABATABAEE et al., 2017 [20] (Iran)
	71 patients of NAFLD; 20-50 years old; both sex	GTE (31.4% EGCG), capsules (500mg GTE), 1 capsule/day	Microcrystalline cellulose	12 weeks	↓BMI, ↓ALT, ↓AST	PEZESHKI et al., 2016 [21] (Iran)
Without NAFLD Double-blind, placebo-controlled, randomized	1021 healthy postmenopausal women; 59.9 years old	GTE (210 mg EGCG), capsules (500mg GTE), 4 capsule/day	Maltodextrin, cellulose and magnesium stearate	12 months	↑ALT, ↓AST 5.1% women in GTE developed moderate or more severe abnormalities in any liver function measure during the intervention period	YU et al., 2017[36] (USA)
	937 postmenopausal women with cancer risk; 60 years old	GTE (210 mg EGCG), capsules (500mg GTE), 4 capsule/day	Maltodextrin, cellulose and magnesium stearate	12 months	Women on GTE reported significantly higher incidence of nausea and dermatologic AEs, and significantly lower diarrhea incidence. GTE group (6.7%) experienced an alanine aminotransferase (ALT) elevation compared with placebo group	DOSTAL et al., 2015 [37] (USA)
	60 mildly hypercholesterolemic patients; 35-55 years old; both sex	GT drink (128.4 mg EGCG) 600 mL/day	Tea flavor drink (with very less concentration of catechin)	12 weeks	A slight decrease in the levels of AST and ALT in all groups (include placebo), but no significant changes were noted. Out of 60 patients, three patients were diagnosed with mild fatty liver (during the baseline). Upon consumption of CEGT completely reversed the fatty liver to normal hepatic condition.	VENKATAKRISHNAN et al., 2018[35] (Republic of China)

NOTE: ALT: Alanine transaminase; AST: Aspartate aminotransferase; BMI: Body Mass Index; EGCG: Epigallocatechin-3 Gallate; GTE: green tea extracts; GTP: Green tea polyphenols; HFD: high-fat diet;.

Table 2 - Characteristics, methodological aspects and main results of the animal studies included in systematic review

Experimental model	Animal	Characteristics of induce/Diet	Characteristics of green tea intake	Main outcomes	Reference/ Quality
Induce → Intervention	Sprague-Dawley rats ♂	HFD (60% fat), 16 weeks	0.32% EGCG, in diet, 16 weeks	↓TG- H ↓TLR4 signaling - protein expression Improved the expression levels of all of markers	HOU et al., 2020[22] (16/20)
	C57BL/6 mice ♂	HFD (34% fat and 5.31 kcal/g), 4 weeks	GTE by gavage (500 mg/kg), 12 weeks	↑ Hepatic glycogen depot ↓macrovesicular steatosis, TG, and cholesterol content ↓Markers of inflammation ↑Genes related to catabolism, lipolysis and β-oxidation pathway ↑ ↓ Genes involved in TG accumulation ↓miRNA 34 and ↑ miR-194	TORRES et al., 2019[28] (17/20)
	C57BL/6J mice ♂	HFD (D12492 - 60% fat - lard), 12 weeks	GTE (30% total catechins,48% EGCG), in diet (2%), 8 weeks	Improvement of hepatic metabolites implicated in dysregulated lipid metabolism, insulin resistance, and inflammation. ↑Amino acids↑ and B-vitamin metabolites	SASAKI et al., 2019[29] (18/20)
	C57BL/6 mice ♂	HFD (D12492 - 60% fat), 12 weeks	GTE (30% total catechins, 48% EGCG), in diet (2%) 8 weeks	↓ALT, ↓MDA-H, ↓ Hepatic protein ↓Liver lipid accumulation ↓; ↓ Histological and biochemical evidence of liver injury; ↓ Hepatic mRNA expression of TNFR1 and its adaptor proteins	LI et al., 2017[7] (15/20)
	Zucker fatty rats ♂	HFD (59% fat), 2 weeks	GTP (98% purity) by gavage (200 mg/kg), 8 weeks	↓ALT , ↓AST, ↓TG-H ↓ Expression of enzymes lipogenesis pathway	TAN et al., 2017[30] (15/20)
	Wistar rats ♂	Cafeteria diet (58% fat) 8 weeks	GTE (30% total catechins) by gavage (500 mg/kg) 12 weeks	AST =, ALT = GT treatment was effective in reducing the content of both lipids in the liver of the animals	ROCHA et al., 2016[31] (17/20)
HFD + Green Tea **Simultaneous**	C57BL/6J mice ♂	HFD (402400 - 61.3% fat),	GTE in diet (2%), 35 weeks	↓ALT , ↓AST, Inhibited lipid accumulation, decreased proliferation, induced apoptosis	COIA et al., 2020[32] (17/20)
	Wistar rats ♂	HFD (diet modified 50% fat - Lard and Sunflower oil)	GTE in diet (1.1% and 2%), 8 weeks	↓Amount of fat in the livers, adding GTE at 1.1% and 2.0% A higher percentage of GTE in the diet caused an even more effective decrease in lipid droplets in hepatocytes	KAROLCKAK et al., 2019[33] (15/20)

	C57BL/6J mice ♂	HFD (AIN-76- 51.9% fat - lard and corn oil)	GTE (77%total catechins, 55%EGCG), in diet (0.4%) 4 weeks	Hepatokine production: ↓LECT2, ↓SeP Phosphorylation of: =AMPK, ↓eIF2α	ONISH et al., 2018[34] (16/20)
	C57BL/6 mice ♂	HFD (60% fat)	GTE by gavage (30, 60, and 120 mg/kg), 12 weeks	↓TG- H (60 and 120 mg/kg GTE) Protein levels lipogenesis-related genes: p-ACC↓, FAS↓, SCD-1↓, SREBP-1↓, Sirt1↑, p-AMPK↑, AMPK= and ACC=	BAE et al., 2018[23] (14/20)
	C57BL/6J mice ♂	HFD (TP230100 - 45% fat)	EGCG by drinking water (2 $g.L^{-1}$), 16 weeks	EGCG supplement significantly ameliorated oxidative stress by up-regulating the IRS-1/AKT and Keap1/Nrf2 transcriptional pathways	MI et al., 2018[24] (14/20)
	C57BL/6J mice ♂	HFD (60% fat)	EGCG (94.5% purity) in diet (0.32%), 33 weeks	Improved fatty liver after treatment for 17 weeks (but effects less apparent at week 33) ↑ mRNA levels of CYP7A1, HMG-CoA reductase, LDL-R, and SCARB1	HUANG et al. , 2018[25] (15/20)
	C57BL/6J mice ♂	HFD (TD06414- 60% fat)	GTE (40.5% total catechins, 21.3% EGCG) in diet (0.25%), 12 weeks	LM ↓, TG-H ↓, COL-H ↓ Observed a reduction of the accumulation of hepatic lipid droplets. Recovered levels of LPLs (such as lysophosphatidylcholine, lysophosphatidylethanolamine and lysophosphatidylserine)	NAN et al., 2018[6] (15/20)
	C57BL/6J mice ♂	HFD (TD06414- 60% fat)	GTE (40.5% total catechins, 21.3% EGCG) in diet (0.25%), 12 weeks	↓ALT , ↓AST, ↓HMGCR, Ameliorated hepatic steatosis	CHOI et al., 2016[5] (16/20)
	Swiss mice ♂	HFD (AIN-93 modified 50% fat)	GTE (50 mg/kg) by gavage, GTE contain 98% EGCG 16 weeks	↓VLDL-TG Protein expression: ↑AdipoR2, ↑SIRT1, ↑pLKB1, ↑pAMPK, ↓ACC, ↓SREBP-1, and ↓ChREBP	SANTAMARINA et al., 2015[26] (17/20)
	C57BL/6 mice ♂	HFD (D12492- 60% fat)	GTE (48% EGCG) in diet (0.5%, 1.0%) and CC (184 mg EGCG) in diet (0.4%), 11 weeks	↓ALT , ↓AST, ↑CPT-1, ↑ACAA2, ↓CPT-2 and ↓ACAD Mitochondrial β-oxidation was moderated by the consumption of GT	LEE et al., 2015[27] (15/20)
Healthy animals	ICR mice ♂	-	EGCG in diet (0.05%, 0.75% or 1.5%) 2, 7 or 10 days	↓CYP3A, ↓translocation of PXR to the nucleus	IKARASHI et al., 2017[38] (17/20)

	C57BL/6J mice ♂	-	EGCG (93% purity) by gavage (250, 500 or 750 mg / kg, ig.) 3 days	Dose of 500 - 750 mg/kg- ↑ALT, ↑hepatic inflammation, necrosis, and hemorrhage	JAMES et al., 2017[39] (15/20)
	Albino Westar rats ♂	-	GTE by gavage (250, 500 or 1000 mg/kg/day) 12 weeks	Dose of 250 mg/kg/day seemed to be safe; doses of 500 mg/kg/day and 1000 mg/kg/day had deleterious effect (more evident in the latter dose) The central veins and hepatic sinusoids were congested, hepatocytes degenerated, hypertrophy of the hepatic arteries, and cellular infiltration	ZAKI et al., 2017[40] (16/20)
	Zucker fatty rats ♂	-	GP (75% total catechins, 57% EGCG) in diet (0.1% and 3%), 4 weeks	↓CYP3A, ↑CYP2C, =CYP1A, =CYP2D, and =CYP2E No changes were found in the hepatic CYP3A levels in mice receiving a diet containing 0.1% GP	IKARASHI et al., 2016[41] (16/20)

NOTE: ACAA2: A 3-cetoacil-CoA tiolase; ACAD: Acyl-CoA dehydrogenase; ACC: Acetyl-CoA carboxylase; AdipoR2: Adiponectin Receptor 2; AKT: Protein kinase B; ALT: Alanine transaminase; AMPK: AMP-activated protein kinase; AST: Aspartate aminotransferase; ChREBP: Carbohydrate-responsive element-binding protein; CPT-2: Carnitine palmitoyltransferase 2; CYP1A: Cytochrome P450, family 1, subfamily A, polypeptide 1; CYP2C: Cytochrome P450 Family 2 Subfamily C ; CYP2D: Cytochrome P450 Family 2 Subfamily D; CYP2E: Cytochrome P450 Family 2 Subfamily E; CYP3A: Cytochrome P450 Family 3 Subfamily A ; EGCG: Epigallocatechin-3 Gallate; eIF2α: Eukaryotic translation initiation factor 2 subunit alpha; FAS: apoptosis antigen 1 GTE: green tea extracts; GTP: Green tea polyphenols; HFD: high-fat diet; IRS-1: Insulin receptor substrate 1; MDA-H: MDA hepatic; TG-H: Triglycerides hepatic.; LM: Liver Mass; COL-H: hepatic cholesterol

As for experimental studies, two studies used GTE[37,38], and two studies used EGCG[35,36]. The dose of GTE used ranged from 250mg / day to 1000mg / day. The duration of the experiment varied from 3 days to 12 weeks of green tea administration. The main outcomes found were: hepatic inflammation[36], congestion of the central veins and hepatic sinusoids, hypertrophy of the hepatic arteries, and cellular infiltration (when administered a dose of 500-1000 mg)[37]; decreased levels of CYP3A[35,38]; and reduction of PXR translocation to the nucleus[35] (Table 2).

3.4 DISCUSSION

In most of the studies covered in this systematic review, green tea has benefits for liver function, both in the presence and in the absence of NASH. The use of green tea was able to decrease the body mass index, reduce the serum levels of ALT, AST, reduce the hepatic levels of triglycerides and cholesterol, as well as improve the liver histomorphometric aspects. However, in 15% of the applied studies, the use of green tea caused hepatoxicity, culminating in centrilobular necrosis, interstitial hemorrhage, and inflammatory cell infiltrations composed mainly of mononuclear cells.

3.4.1 THERAPEUTIC EFFECT OF GREEN TEA ON THE HEPATIC FUNCTION OF INDIVIDUALS (AND ANIMALS) WITH NAFLD

All human studies that have evaluated the therapeutic effect of green tea on NAFLD patients have occurred in an Asian population and lasted for twelve weeks[16,17]. It is worth noting that the population's food consumption differs greatly between continents[39–41]. In North America, it is common to consume high-fat foods and low consumption of fruits and vegetables[40]. In Asian countries, it is common to consume protein and vegetables[39,41]. Considering this difference in the dietary pattern between countries, we believe that the therapeutic effect of green tea can be different if we evaluate a population that is in another geographical dimension. Therefore, it is necessary to carry out studies in humans in different variations to identify its real effect and potential.

In human studies in NAFLD patients, regardless of the daily dose of green tea extract (384 to 1000 mg) was a significant decrease in body mass and liver enzymes (ALT and AST)[16–18]. Only in the study by Tabatabaee et al.[17], which used a dose of 384 mg/day, the decrease in ALT was not significant. Also, in the study proposed by Hussain et al.[16] beside the transaminase assessment, there was an assessment of the fatty liver classification through abdominal ultrasound. It was identified that the GTE (1000mg / day) caused a 67.5% regression of fatty liver changes[16].

The physiological mechanism and metabolic pathways involved in reducing BMI and improving liver function through the use of green tea are still being investigated. From the experimental data collected by this review, we identified that the consumption of green tea is associated with decreased liver fat and the expression of catabolism genes[5,7,23,25,26,30,31]. It is believed that polyphenols can modulate the expression of several mRNAs, and these mRNAs regulate several biological activities[26,42]. According to the mechanism proposed by Torres et al.[26], the GT acts in the epigenetic regulation of miR-34a and miR-194, and these mRNAs, control the expression of genes involved in the accumulation of hepatic TG, catabolism, lipolysis and β-oxidation[26].

Increased hepatic synthesis and TG accumulation, reduced hepatic mitochondrial β-oxidation, and inadequate secretion of VLDL into the blood contribute to the accumulation of lipids in the liver[43]. In the experimental studies collected, it was observed that the consumption of GT decreased the expression of genes involved in TG accumulation (apoA5 and Cd36)[26] and, consequently, decreased the accumulation of liver lipids[7,20,25,26,28,29]. In the study by Nan et al.[6], The use of 0.25% GTE in the diet for 12 weeks resulted in reduced levels of TG and hepatic cholesterol (i.e. observed by the analysis of hepatic lipid extraction), and reduced hepatic lipid accumulation (i.e. observed through histological analysis).

Also, it was shown that the GT can act on hepatic mitochondrial β-oxidation. An increase in expression was observed in the genes related to catabolism (AdipoR1 and AdipoR2)[23,26] and β-oxidation (SIRT1, pLKB1, pAMPK)[20,23,26,28] and decrease in protein expression SREBP-1 and ChREBP[20,23,26,28]. In the study proposed by Santamarina et al.[23], 50mg / kg of GTE, administered by gavage for 12 weeks, was able to promote an increase in the gene expression of AdipoR2, SIRT1, pLKB1, and a decrease in the gene expression of SREBP-1 and ChREBP.

3.4.2 EFFECT OF GREEN TEA ON THE HEPATIC FUNCTION OF INDIVIDUALS (AND ANIMALS) WITHOUT NAFLD

Of the three human studies that evaluated the effect of green tea on individuals who did not have NAFLD, two had harmful outcomes for liver function[33,34]. In studies that used a high daily dose of GTE (2000mg), patients showed a significant increase in ALT[33,34]. In the study proposed by Yu et al.[33], patients presented a decrease in AST followed by an increase in ALT. In the same experiment, about 5.1% of women developed moderate and severe liver function abnormalities[33]. In the study proposed by Venkatakrishnan et al.[32], patients consumed 600mL of green tea drink per day (twelve weeks). All patients (i.e. including the placebo group) had non-significant decreases in ALT and AST.

As for the experimental studies systematically included in this review, the majority showed damage to liver function [35–38]. The higher the dose administered, the greater the liver damage. The main outcomes found in these studies were: congestion of the central veins and hepatic sinusoids[36,37], hypertrophy of the hepatic arteries and cellular infiltration (when administered a dose of 500-1000 mg)[37]; decreased levels of CYP3A[35,38]; and reduction of PXR translocation to the nucleus, aspects related to hepatoxicity[35].

This hepatoxicity is related to functional and/or structural changes in the liver. In the article by Chan et al. [44], hepatoxicity was confirmed by the decreased body weight gain and liver histopathological lesions. The rats showed both increased mortality and weight ratios between the liver and body. Changes in the liver were considered the primary toxic effects of GTE, while changes in other organs were secondary to the effects associated with high dose-related to stress. In the same study by Chan et al.[44], several doses of GTE (0-1000mg/kg) were tested and the observed effect was the higher the GTE dose, the greater the liver damage[44].

In the study by Zack et al.[37] a subchronic administration of GTE for 12 weeks resulted in damage to the structure and function of the rat's liver. The 250mg/kg/day dose of GTE did not cause serious damage. However, when GTE was administered in higher doses (500 and 1000mg) was a more evident deleterious effect, with degeneration of hepatocytes and cell infiltration[37].

In the Ikaraski et al.[38] study, the rats were fed a diet containing 3% GP (i.e. with 75% catechin) for 4 weeks. In this experiment, was a decrease in the expression of CYP3A, and consequently a decrease in its metabolic activity. However, in diets with a low GP (0.1%) dose, no changes in the levels of CYP3A gene expression were identified[38]. This enzyme (CYP3A) participates in the metabolism of drugs and toxins[35,38].

The catechins present in green tea are the main metabolites that favor hepatoxicity[45,46]. These catechins can have a genotoxic or carcinogenic effect when administered in high doses or over a long period[46–48]. When using the infusion of green tea, toxicity is practically non-existent, unlike what happens when using the extract or the isolated polyphenol[46].

Although this systematic review is complete and comprehensive, some limitations must be considered. Our objective was to carry out a current review, so we limit our systematic search for articles published in the last 5 years, however, this delimitation may exclude some interesting studies. Besides that, some studies do not report how green tea resources are used or the polyphenols content, which makes it difficult to discuss the work in this case.

3.5 CONCLUSION

Therefore, considering the studies evaluated in this systematic review, it is observed that the use of green tea proved to be efficient as a treatment for NAFLD, showing improvement in liver function in human and experimental studies. Doses between 500 and 1000 mg per day of GTE, administered for 12 weeks in patients with NAFLD, brought benefits to liver function. However, it is necessary to evaluate the dose and time of administration of green tea, especially in healthy patients, in order not to cause liver damage. Doses above 2000 mg/day of GTE in healthy patients brought harmful outcomes to liver function. Therefore, further studies are needed to better investigate the therapeutic potential of green tea.

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4. CAPÍTULO II - EFFECT OF THE GREEN TEA INFUSION ON THE METABOLIC AND HEPATIC MODIFICATIONS OF MICE FED A HIGH-FAT DIET

ABSTRACT

Background: The study aims was to evaluate the effect of the green tea infusion on the metabolic and hepatic modifications of mice fed a high-fat diet. Male C57BL/6 mice were randomly allocated into 4 groups: Male C57BL / 6 mice, aged between forty and sixty days, were randomly divided into four groups: control (C, n = 8), control + green tea (CGT, n = 9), high-fat diet (HFD, n = 9) and high fat diet + green tea (HFDGT, n = 9). The diet and water (or green tea) were offered ad libitum to the animals for 16 weeks. The following parameters were evaluated in all experimental groups: BMI (Body Mass Index) (every two weeks); fasting blood glucose (at the beginning and in the final experiment); response to TTGO - Oral Glucose Tolerance Test (15 weeks old); TTI - Insulin Tolerance Test (16 weeks old); and determination of the lipid profile and transaminases (at 16 weeks). The animals in the HFD group, when compared to the animals in group C, had higher final body weight (36.00 ± 1.25 g vs 29.87 ± 1.08 g, $p \leq 0.001$), greater relative liver weight (5 , 48 ± 0.40 g vs 3.71 ± 0.25 g, $p \leq 0.001$), increased fasting blood glucose (202.00 ± 9.78 mg / dl vs 142.83 ± 4.21 mg / dl, $p \leq 0.001$). In addition, the HFD group presented other disorders such as glucose intolerance ($p \leq 0.05$) and peripheral insulin resistance ($p \leq 0.001$) evidenced by the TTGO and TTI Area Under Curve (ASC) values. The HFDGT group had lower final body weight (32.55 ± 1.03 g vs 36.00 ± 1.25 g, $p \leq 0.05$), decreased fasting glucose (162, 33 ± 8.97 mg / dl vs 202.00 ± 9.78 mg / dl $p \leq 0.01$), lower serum levels of HDL cholesterol ($p \leq 0.001$) and total cholesterol ($p \leq 0.001$) when compared to the HFD group. In the HFDGT group, green tea was effective in improvement in glucose sensitivity ($p \leq 0.05$), but did not have a significant impact on TTI values. There were no significant changes in serum levels of AST and ALT among the interest groups. Green tea contributed to the improvement of anthropometric parameters and glucose metabolism, but there were no significant changes in transaminases.

Keywords: *Camellia sinensis,* liver function, hepatic steatosis, transaminase.

4.1 INTRODUCTION

Obesity is a disorder caused by an excessively fat-rich dietary intake associated with a lack of physical exercise[1]. The obesity prevalence has increased dramatically in recent years and is a worldwide health problem[2,3]. Obesity is a risk factor for numerous diseases, such as type 2 diabetes mellitus, metabolic diseases, and hepatic steatosis[4.]

Hepatic steatosis is characterized by the accumulation of lipids in the liver, mainly triglycerides, in amounts greater than 5% in hepatocytes[4]. For treatment, previous studies and the ESPEN 2019[5] guideline, it recommends a 7 to 10% weight loss to improve steatosis[6,7]. However, obese people report difficulties in adhering to new eating habits[3] and new strategies for weight loss should be investigated. Therefore, obesity prevention has become a central issue in food and molecular nutrition research.

Green tea is obtained from the drying of the *Camellia sinensis* leaves, which concentrates methylxanthines (mainly caffeine) and phenolic compounds such as catechins (epicatechin 3-gallate, epigallocatechin, epicatechin and epigallocatechin-3-gallate) as the main chemical compounds [8,9]. The biological effects in vitro of green tea extract were assessed [10,11]. In the study proposed by Cho et al. [12], green tea showed benefits for the regulation of lipid metabolism in vitro. In cultured cells, green tea was able to inhibit adipogenesis and lipogenesis, and favor the oxidation of fatty acids[12].

In addition, there is evidence that confirm the benefits of green tea in improving fatty liver. In the study by Torres et al.[13], the administration of 500 mg/kg/day in mice fed with HFD (high-fat diet), for 12 weeks, was able to decrease the infiltration of TG and hepatic cholesterol. Moreover, in the study proposed by Coia et al.[14], mice fed HFD and GTE (2% in diet) simultaneously for 35 weeks, had lower serum levels of transaminases (ALT and AST) and less accumulation of liver lipids when compared to mice fed exclusively with HFD.

It is noted that most of the investigated studies use the GTE. Therefore, it is necessary to investigate the biological effects of green tea consumption after being prepared as an infusion in a mice model. Considering the facts explained, the aim of this study is to evaluate the green tea infusion effect on the metabolic and hepatic modifications of mice fed a high-fat diet.

4.2 MATERIAL AND METHODS

4.2.1 GREEN TEA INFUSION PREPARATION

Green tea (GT) was obtained commercially at Viva Natureza Produtos Naturais LTDA and prepared as previously described by Hajiaghaalipour et al.[15]. Briefly, 2 g of green tea leaves fragments were immersed for 5 min in 100 mL boiling water; then, the hot GT solution was filtered to remove solid residues and cooled to room temperature.

4.2.2 ANIMAL STUDIES

All experimental procedures were according to the Guidelines for the Care and Use of Laboratory Animals, and as approved by the Ethics Committee for the Use of Experimental Animals of the School of Veterinary Medicine and Zootechny, Federal University of Bahia (CEUA/EMEVZ-UFBA; under the Protocol number: 03/2019).

Male C57BL/6 mice, aged forty to sixty days, were obtained from the Gonçalo Moniz Research Center-FIOCRUZ (Salvador, Bahia, Brazil) breeding. The animals, weighing approximately 22 ± 2g, were housed in temperature-controlled rooms, 22– 25°C, under a 12 h light-dark cycle, with ad libitum access to water and food.

4.2.3 EXPERIMENTAL DESIGN

The animals were separated into four experimental groups: control (C), control + green tea (CGT), high-fat diet (HFD), and high-fat diet + green tea (HFDGT). The groups and detailed study plans are represented in Figure 1. The control diet (Pragsoluções Biociências) consisted of 16.4% protein, 10.5% fat, and 73.1% carbohydrate with adequate mineral and vitamin contents. The HFD (Pragsoluções Biociências) consisted of 17.5% protein, 57.9% fat, and 24.7% carbohydrate with adequate mineral and vitamin contents (Table 1). The tea beverages were the sole source of drinking fluid in the tea groups. All drinking fluid was given to rats in bottles, which were replaced daily with freshwater or tea.

Table 1 - Composition of the control diet and the high fat diet

High-Fat Diet			Control Diet		
	g%	KCal		g%	KCal
Protein	23.4	17.5	Protein	16.8	16.4
Carbohydrate	33.2	24.69	Carbohydrate	74.3	73.1
Fat	34.6	57.9	Fat	4.8	10.5
Total Kcal/g	3.87	100	Total Kcal/g	4.07	100
Ingredients	g	Kcal	Ingredients	g	Kcal
Sucrose	10.0	400	Maltodextrin	170	690
Soybean oil	8.6	774	Sucrose	0	0
Lard	24.7	2223	Soybean oil	25	225
Mineral mix	4.6	0	Mineral mix	10	40

Adapted from DALTRO et al.[16]

At the end of the 16-week experimental period, the animals were euthanized by decapitation. From each animal, blood samples were collected, and the hepatic tissue was dissected out and then weighed. The serum was separated from the total blood and was stored at −80°C until analysis.

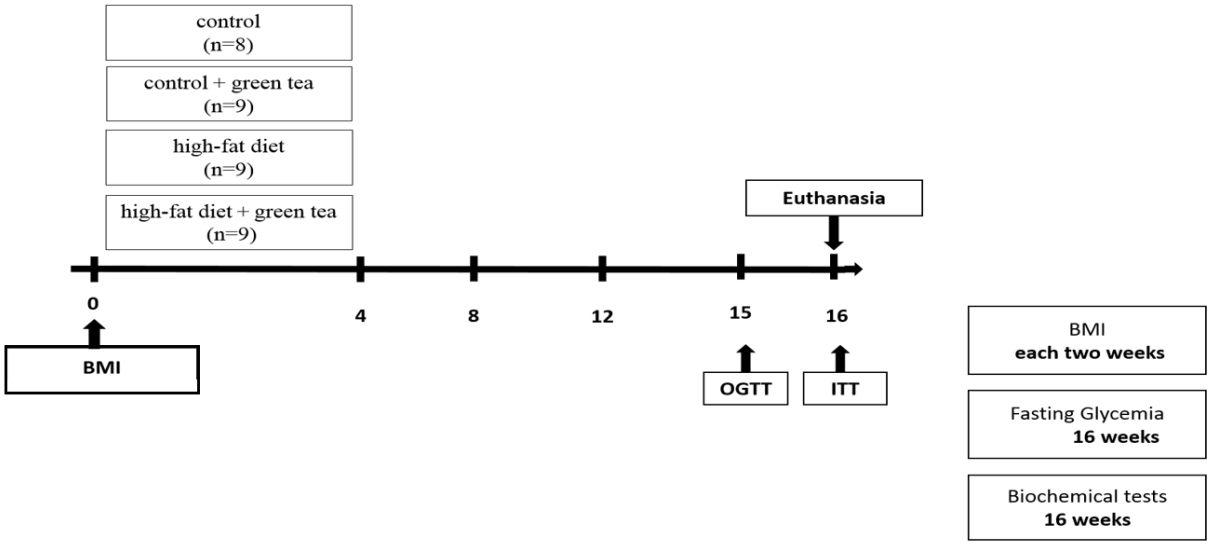

Figure 1 - Experimental design: the figure depicts the grouping and study carried out. Mice were randomly divided into 4 groups as the following: Group C (Control) received standard diet and autoclaved water as a sole drinking source. Group CGT (control + green tea) received a standard diet and green tea as a sole drinking source. Group HFD (high-fat diet) received a high-fat diet and autoclaved water as a sole drinking source. Group HFDGT (high-fat diet + green tea) received a high-fat diet and green tea as a sole drinking source. The following parameters were evaluated in mice from all experimental groups: the BMI (every two weeks), the fasting glycemia (final experiment), and response to the Oral Glucose Tolerance Test (OGTT, 15 weeks), and Insulin Tolerance Test (ITT, 16 weeks), and biochemical tests (16 weeks).

4.2.4 BODY MASS INDEX AND RELATIVE WEIGHT OF ORGANS

The animal's body weight and length (anus to mouth) were measured in all groups every two weeks. The Body Mass Index (BMI) was calculated using the following formula [17]:

$$BMI = \text{body weight (g)/length (cm}^2\text{)}$$

The liver relative weight (liver weight/animal weight x 100) was measured after euthanizing the animals. The data measurements are expressed in percentage weight ± standard error of the mean (SEM).

4.2.5 ASSESSMENT OF FASTING BLOOD GLUCOSE, ORAL GLUCOSE TOLERANCE TEST, AND INTRAPERITONEAL INSULIN TOLERANCE TEST

The fasting blood glucose was performed before and at the experiment's conclusion. The mice were overnight fasted before screening for fasting blood glucose (8 hours). Blood glucose levels were determined in blood samples from the tail vein by using the On Call® Plus II glucose sticks. The results were obtained as milligrams per deciliter of blood [18].

The Oral Glucose Tolerance Test (OGTT) was performed on the experiment conclusion. The mice were fasted before screening for glycemia (6 hours). The glucose solution (i.e. 1 g glucose/kg body weight) was administered directly into the stomach using a feeding needle (gavage). The blood sample for glucose level measurements was obtained from a tail vein

incision, before and after 15, 30, 60, and 120 min of glucose administration. Blood glucose was measured by using a glucometer (On Call® Plus II) and the obtained results are shown as milligrams per deciliter of total blood [18].

Insulin sensitivity was assessed in all groups by Insulin Tolerance Test (ITT). Insulin (i.e. 0.75 U insulin/kg body weight) was injected intraperitoneally to animals after a fasting period of three hours. Blood was sampled by a tail vein incision for glucose level measurements before and after 15, 30, 45, and 60 min of insulin administration. The blood glucose was measured as the same as the above described [18].

4.2.6 MEASUREMENT OF SERUM LIPID PROFILE AND LIVER ENZYMES

At the end of the 16-weeks, after a fasting period of 12 h, blood samples were collected, placed in microtubes, and centrifuged at 1000 rpm at 4°C for 10 min to obtain blood plasma, which was stored at −80°C for further analysis.

The plasma lipid profile and liver enzymes were measured in automated equipment ADVIA 1800® Clinical Chemistry System (Siemens®). The plasma lipid profile was measured as total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglycerides (TG) while the liver function was obtained through the measurements of aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

4.2.7 STATISTICAL ANALYSIS

Every effort was made to minimize the number of animals used and to avoid any discomfort. The sample size was determined using the *Winpepi* program (version 1.69). The null hypothesis was tested with a power of at least 0.99, considering a habitual loss rate of 15%. A 95% confidence interval with a 5% critical level was accepted to admit significance. The sample size calculation resulted in 35 animals, representing minimally eight or nine animals per group.

Data analysis was performed in two moments. First, the tests were performed to detect Outlier (i.e. Grubb's test) and produce summary measures. Then, the comparative analysis was performed to check for the significant differences among groups (i.e. ANOVA's test). The analysis of variance (ANOVA) was performed, followed by Tukey post-test. The differences among groups were considered significant when $p < 0.05$, for a confidence interval of 95%. The data were shown as mean ± SEM. Statistical analysis was performed with GraphPad Prism 5.01 for Windows (GraphPad Software. Inc. CA.USA).

4.3 RESULTS

4.3.1 THE EFFECTS OF GREEN TEA INFUSION ON BODY WEIGHT, BMI, AND LIVER WEIGHT

At the termination of a 16 week experiment period, the obtained data shows mice fed with HFD when compared with those fed with the C diet had significantly higher final body weight ($p < 0.001$), relative liver weight ($p < 0.001$), and fasting glycemia ($p < 0.001$). Therefore, the consumption of HFD for a 16 weeks period induced body and glycemia changes in these mice (Table 2).

Table 2 – Body weight, relative liver weight and glycemia of the experimental groups

Parameters	C (n=8)	CGT (n=9)	HFD (n=9)	HFDGT (n=9)
Initial Body Weight (g)	25.37 ± 0.56	26.11 ± 0.58	27.01 ±0.76	25.55 ± 0.94
Final Body Weigh (g)	29.87 ± 1.08	29.77 ± 0.43	36.00 ± 1.25***	32.55 ± 1.03$^{\#}$
Relative liver weight (%)	3.71 ± 0,25	3.82 ± 0.19	5.48 ± 0.40***	4.81 ± 0.14
Glycemia (mg/dL)	142.83 ± 4.21	170.33 ± 7.14	202.00 ± 9.78***	162.33 ± 8.97**

C- control; CGT - control + green tea; HFD- high-fat diet; HFDGT - high-fat diet + green tea; Data were expressed as mean ± standard error of mean. ANOVA and Tukey post-test was used to study the effects of diet; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus C; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ versus HF.

The HFDGT group showed final body weight ($p<0.05$) and glycemia ($p<0.01$) significantly lower when compared to the HFD group, but, on the other hand, the relative liver weight did not alter significantly (Table 2). Therefore, the consumption of green tea decreased the speed of weight gain and the increase in mice glycemia fed with a high-fat diet. However, the comparison between the C and CGT groups was not significantly altered.

Mice fed with the HFD when compared to those fed with the C diet showed statistically significant BMI increase after 6 weeks of experiment. However, The HFDGT group when compared to those fed with the HFD group showed statistically significant BMI increase only by 6 and 16 weeks (Figure 2) There was no significant difference in BMI between groups C and CGT.

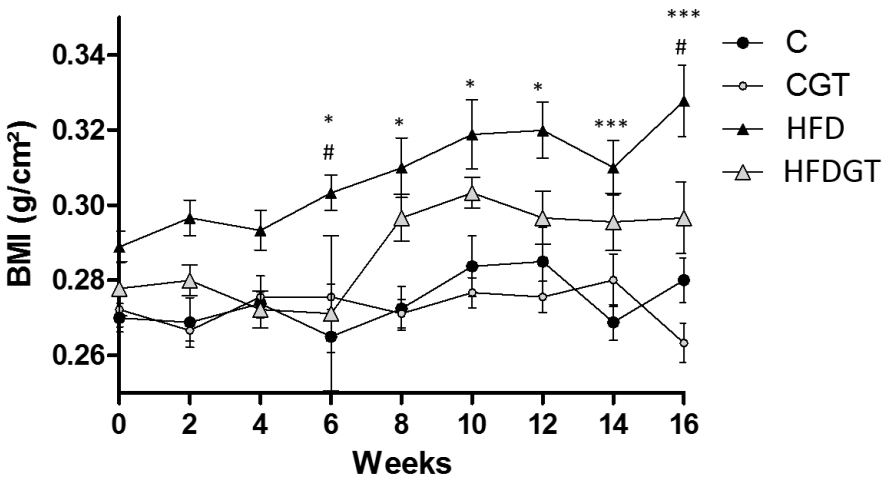

Figure 2 - BMI was measured in all the groups every two weeks. Values are expressed as mean ± SEM; experiments were taken with 8 to 9 mice per group. ANOVA and Tukey post-test was used to study the effects of diet The mice C diet versus HFD, ***$p \leq 0.001$; *$p \leq 0.05$. The mice HFD versus HFDGT; # $p \leq 0.05$. Note: control (C), control + green tea (CGT), high-fat diet (HFD), and high-fat diet + green tea (HFDGT)

4.3.2 DIET TYPE EFFECTS ON ORAL GLUCOSE TOLERANCE, AND INSULIN TOLERANCE TESTS

The mice fed the HFD when compared to those fed the C diet showed metabolic disturbances such as glucose intolerance ($p \leq 0.05$), and peripheral insulin resistance ($p \leq 0.001$). The induction of glucose intolerance was evidenced by OGTT and ITT AUC values (Figures 3).

On the HFDGT group, green tea was effective in improvement in glucose sensitivity ($p \leq 0.05$) but did not have a significant impact on ITT values. In addition, we didn't find significant differences between the C and CGT groups (Figures 3).

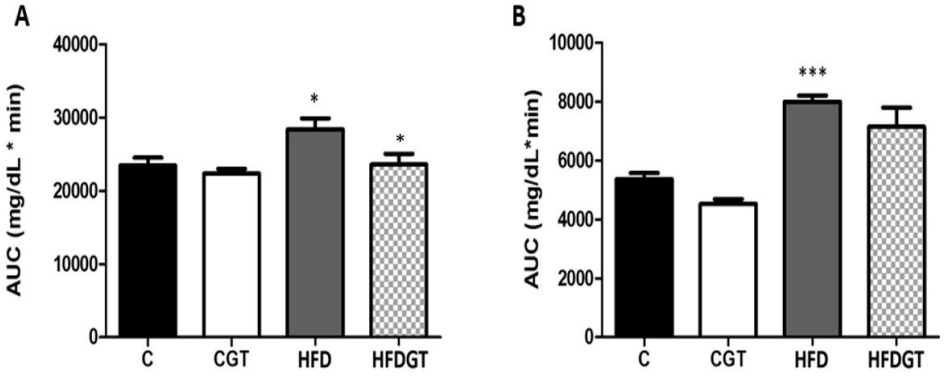

Figure 3 – (**A**) The oral glucose tolerance test. (**B**)The insulin tolerance test. Values are expressed as mean ± SEM; experiments were taken with 8 to 9 mice per group. ANOVA and Tukey post-test was used to study the effects of diet. The mice C diet versus HFD $^{***}p \leq 0.001$,$^{*}p \leq 0.05$. The mice HFD versus HFDGT; * $p \leq 0.05$. Note: control (C), control + green tea (CGT), high-fat diet (HFD), and high-fat diet + green tea (HFDGT)

4.3.3 DIET TYPE EFFECTS ON SERUM LIPID PROFILE

The levels serum of the triglycerides were lower ($p < 0.01$) in mice fed the HFD (HFD group) when compared to the mice fed with the control diet (C group) (Figure 4).

The effect of green tea on the serum lipid profile was evaluated on the HFDGT group compared to the HFD group. The serum total cholesterol and HDL cholesterol were significantly decreased ($p < 0.001$). However, the levels of serum LDL cholesterol and triglycerides didn't decrease significantly (Figure 4).

On mice from the CGT group, when compared to the C group, the levels of serum total cholesterol ($p<0.05$) and HDL cholesterol ($p<0.01$) values were significantly lower , but on the other hand the triglycerides were higher ($p<0.01$) (Figure 4).

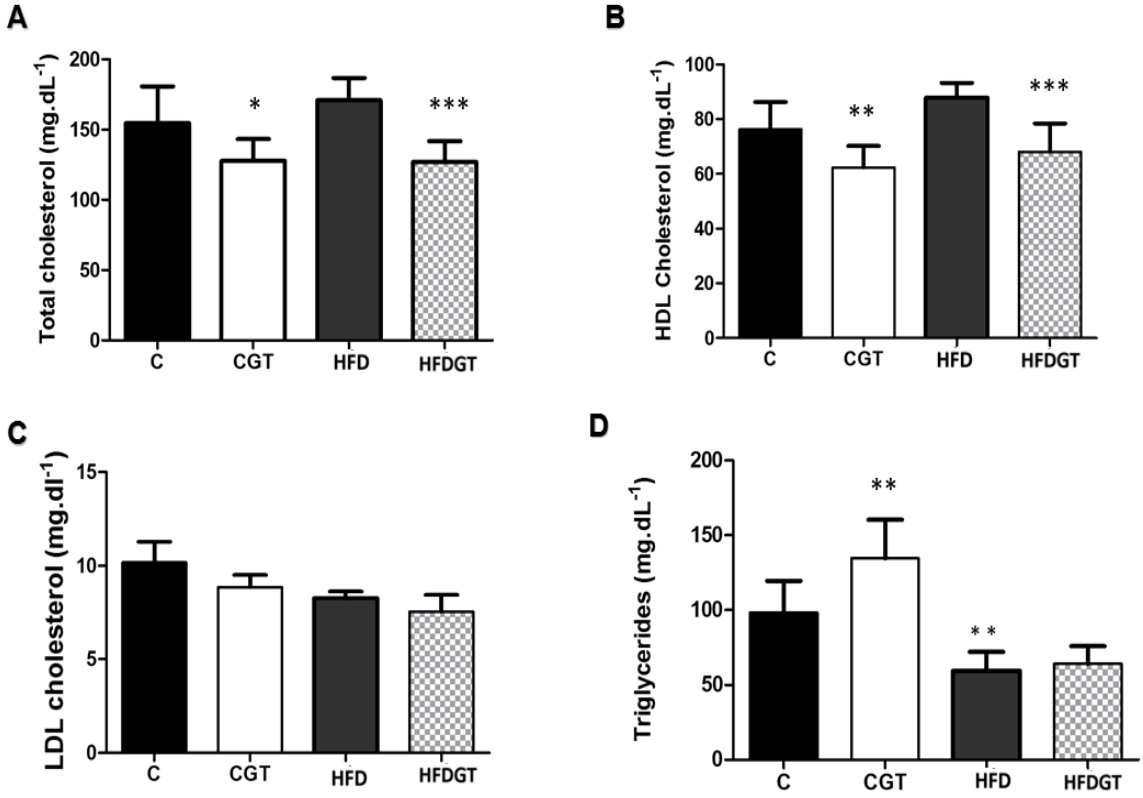

Figure 4 – Serum concentrations of total cholesterol (**A**), HDL cholesterol (**B**), LDL cholesterol (**C**), and Triglycerides (**D**) Values are expressed as mean ± SEM; experiments were taken with 8 to 9 mice per group. ANOVA and Tukey post-test was used to study the effects of diet. The mice C diet versus HFD; $^{**}p \leq 0.01$. The mice HFD versus HFDGT; *** $p \leq 0.001$. The mice C diet versus CGT diet; ** $p \leq 0.01$; $^{*}p \leq 0.05$. Note: control (C), control + green tea (CGT), high-fat diet (HFD), and high-fat diet + green tea (HFDGT)

4.3.4 DIET TYPE EFFECTS ON LIVER ENZYMES

As shown in Figure 5, there was not significant difference in the serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) between groups.

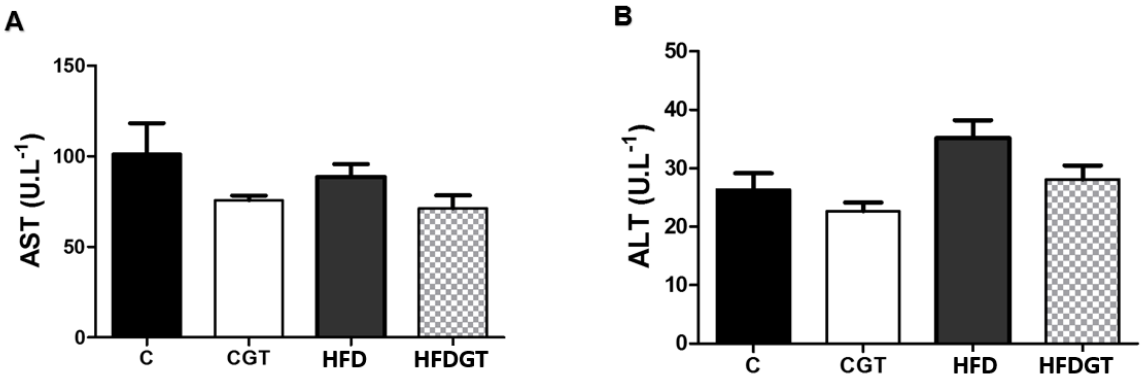

Figure 5 – Serum concentrations aspartate aminotransferase (**A**) and serum alanine aminotransferase (**B**). Values are expressed as mean ± SEM; experiments were taken with 8 to 9 mice per group. ANOVA and Tukey post-test was used to study the effects of diet. Note: control (C), control + green tea (CGT), high-fat diet (HFD), and high-fat diet + green tea (HFDGT)

4.4 DISCUSSION

This study results provide of evidence of green tea consumption in the restoration of overfeed-related dysfunction in mice fed with a high-fat diet, by promoting a lower body weight gain and improving health, as shown by the decrease fasting glucose, the improvement in glucose sensitivity. The mechanisms whereby green tea can influence body weight loss, are an interesting area of investigation.

Previous studies have shown steatosis as the main hepatic consequence of obesity [19,20]. In the present study, the green tea infusion decreased the speed of body weight gain. This may indicate a possible improvement in liver function because, according to current ESPEN[5] recommendations, a 7 to 10% weight loss is recommended to improve steatosis and liver biochemistry[5].

Green tea infusion has also shown to be effective in improving glucose metabolism in mice fed a high-fat diet. These data are similar to previous studies[21–23]. In the study proposed by Xia et al. [21], the Green tea polyphenols promoted a reduction in fasting serum glucose and improved insulin resistance in rats fed with HFD after eight weeks of the experiment. The main pathways involved in the anti-hyperglycemic effect involve: inhibition of the GLUT intestinal system increased insulin-stimulated glucose uptake and decreased the control of gluconeogenesis gene expression [24,25] .

In that study, mice fed with HFD and using green tea infusion had reduced levels of total and HDL cholesterol. In the study proposed by Chen et al.[26], rats fed with HFD and GTE (83% of total catechins) for eight weeks, showed TG, CT, and LDL-c significant decreases, and HDL

increase. Moreover, a study proposed by Dey et al.[27], mice fed HFD and GTE (30% total catechins) for eight weeks, showed TC and TG reductions.

The infusion of green tea did not bring significant changes in serum levels of ALT and AST in mice fed a high-fat diet. In other studies that used GTE (instead of infusing green tea) the results were different. In the study by Choi et al.[30], mice fed with HFD and GTE (30% total catechins) for 12 weeks had reduced levels of transaminases (ALT and AST) when compared to mice fed with HFD. In addition, in the study proposed by Coia et al.[31], Mice fed with HFD and GTE (at 2% in the diet) for 35 weeks, had reduced serum levels of ALT and AST. The catechin content in the extracts is believed to have interfered with the outcomes.

Moreover, although green tea extract offers greater catechin content when compared to infusion[32], in this experiment, green tea prepared as an infusion brought similar benefits to green tea extract, even though it did not have the same amount of catechins as an extract[32]. Perhaps, if the mice had a longer exposure time to the infusion of green tea, other beneficial results could be significant.

In the literature, some mechanisms are suggested to demonstrate the effectiveness of green tea on the liver function when associated with a high-fat diet. The polyphenols present in green tea can inhibit intestinal lipid digestion and absorption[33] and stimulate hepatic lipid metabolism. Besides, these polyphenols can act by promoting thermogenesis, fat oxidation, and fecal lipid excretion [34], contributing to the prevention of adipose tissue expansion[35] and, therefore, can manifest the anti-obesity effect[36].

4.5 CONCLUSION

The general metabolic responses to the infusion of green tea proved to be desirable. In the experimental study carried out, the infusion of green tea was able to decrease the speed of weight gain of the animals, improve the sensitivity to glucose and glycemia. According to current recommendations, the improvement of these parameters are indicative of improved liver function. However, the use of green tea infusion by mice did not significantly change serum levels of transaminases. Therefore, further studies are needed to assess whether a longer period of exposure to green tea infusion can bring other significant benefits.

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4. CONCLUSÕES E PERSPECTIVAS FUTURAS

De acordo com o que foi explanado nessa dissertação, há evidencias de que o chá verde e suas catequinas atuam tanto de forma direta quanto indireta na melhora dos parâmetros antropométricos, metabólicos e hepáticos. Na investigação bibliográfica realizada, foi evidenciado que, as catequinas do chá vede atuam de forma direta na redução dos triglicerídeos do fígado, estimulando a sua secreção pelos hepatócitos. É válido ressaltar que estes estudos utilizaram extrato de chá verde, e não infusão. No estudo experimental realizado nesta pesquisa, a infusão do chá verde foi capaz de melhorar a saúde metabólica e trouxe evidencias de melhora da função hepática. Portanto, o chá verde preparado como infusão traz benefícios similares ao extrato de chá verde, mesmo não possuindo a mesma quantidade de catequinas contidas no extrato.

Visando o referido potencial terapêutico da infusão do chá verde, as novas perspectivas encontram-se na realização de novos testes em animais e humanos. Quanto aos testes em animais, é necessário avaliar se o maior período de exposição à infusão do chá verde (não extrato) pode trazer benefícios significantes para a função metabólica e hepática e se os resultados benéficos podem ser acompanhados de malefícios. Em relação aos estudos em humanos, estes devem ser realizados em diferentes populações, para identificar o verdadeiro efeito da infusão do chá verde sobre a função hepática de indivíduos que consomem dieta com alto teor de gordura.

ANEXOS

ANEXO A – CERTIFICADO DE APROVAÇÃO CEUA

Universidade Federal da Bahia
Escola de Medicina Veterinária e Zootecnia
Comitê de Ética no Uso de Animais
Av. Ademar de Barros, 500 – Ondina-40170-110 Salvador-BA
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CERTIFICADO

Certificamos que a proposta intitulada **"Efeito do consumo regular do chá verde sobre alterações metabólicas, inflamatórias e hepáticas induzidas por dieta hiperlipídica em camundongos"**, registrada com o nº **03/2019**, sob a responsabilidade do(a) **Prof. (a) Ricardo David Couto**, e que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Escola de Medicina Veterinária da Universidade Federal da Bahia, **em reunião de 24.04.2019.**

Finalidade	( )Ensino (X) Pesquisa Científica
Vigência da autorização	24/04/2019 à 18/11/2019
Espécie/linhagem/raça	*Camundongo C57BL/6*
Nº de aninais	116
Peso/Idade	25-35g / 5 meses
Sexo	Macho
Origem	Biotério

Salvador, 25/04/2019.

Prof. Claudio de Oliveira Romão
Coordenador CEUA/EMEVZ-UFBA